Effects of Continuous Exposure of Rats to 100 Per Cent Oxygen at 450 mm. Hg for 64 Days

GERALD A. BROOKSBY, ROBERT L. DENNIS AND ROBERT W. STALEY

Young Sprague-Dawley rats were exposed, under constant uninterrupted conditions, to pure oxygen (99.8 ± 0.2 per cent) at a total pressure of 450 mm. Hg for 64 days and compared with controls. Growth rates and food and water consumption were measured during the exposure period. All animals survived and appeared normal with no signs of distress during the experiment. At the conclusion of the 64-day exposuure period, all animals were sacrificed and histological and hematological studies were performed. There were no significant differences in food and water consumption or demonstrable histological and hematological differences.

It appears that the experimental conditions used were below the threshold level necessary to produce distress or pathology in this strain of rat.

OF PRIME CONCERN for extended space missions are problems which may arise from long-term exposure to pure oxygen or increased oxygen partial pressures. Detailed information is needed regarding prolonged exposures to these conditions.

Previous limited research indicates that the maximum PO_2 man may be able to tolerate lies in the area of 425 mm. Hg;[2,23,30] above this partial pressure pulmonary distress is encountered within several hours.[2,3,8] Animal experimentation, at pressures above a PO_2 of 450 mm. Hg,[10,24] reveals the development of pulmonary damage and in some cases death following relatively short exposures. Several recent studies[14,15,22] indicate the possibility of man's stay below a 425 mm. Hg PO_2, for 30 days, breathing 100 per cent oxygen or oxygen-nitrogen mixtures. Some aspects of man's exposure to 100 per cent oxygen at varying pressures and the difficulties which may be encountered are discussed in the article by Roth.[26]

The threshold level for oxygen damage in mice[9] and rats[10] apparently lies between 550 and 650 mm. Hg PO_2. Campbell,[7] using a variety of animals, found no problems with exposures up to 33 days with oxygen at 433 mm. Hg. Berry and Smythe[5] noted increased urinary nitrogen excretion in mice exposed to 100 per cent oxygen at 226 and 187 mm. Hg for 3-4 weeks, but could not attribute this phenomenon to the pure oxygen environment. Two groups of investigators reported no ill-effects on mice[18] and rats[11] exposed to 51 and 24 days of breathing pure oxygen at 197 mm. Hg.

Due to the limited information available on the mechanism of oxygen toxicity and threshold levels of oxygen tolerance, the authors initiated a series of studies to investigate (1) what is the upper acute threshold level of oxygen pressure an animal can tolerate and (2) what chronic effects are produced by prolonged exposure to a PO_2 slightly below the level where acute damage is reported to occur? This report details the results of the first of this series using a PO_2 of 450 mm. Hg.

MATERIALS AND METHODS

Animals used in this project were 5-6 week-old male, Sprague-Dawley rats with a mean initial weight of 133 g. The animals were obtained from a local supplier* and were observed for at least two weeks in our laboratory before use. Twenty-nine animals were divided into three groups. Groups I and II consisted of 12 and 6 rats, respectively. Each rat was housed in an individual, plexiglass chamber (Figure 1). Group I breathed

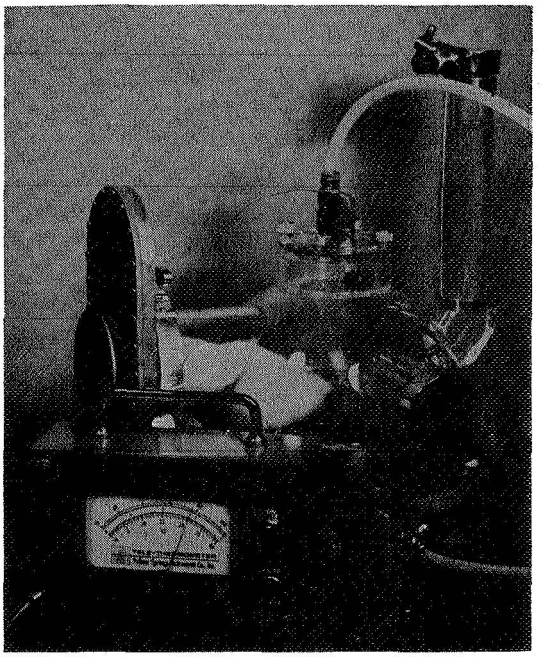

Fig. 1. Chamber for exposing animals to air or pure oxygen for 64 days.

Dr. Brooksby is Research Scientist, NASA-Ames Research Center, Biotechnology Division, Moffett Field, California.

Dr. Dennis is Chief Pathologist, San Jose Hospital, San Jose, California.

Mr. Staley is Engineering Technician, NASA-Ames Research Center, Biotechnology Division, Moffett Field, California.

*Berkeley Pacific Laboratories, Berkeley, California.

Reprinted from *Aerospace Medicine*, Vol. 37, No. 3, March 1966

99.8 ± 0.2 per cent oxygen at a total pressure of 450 ± 5 mm. Hg. Group II was maintained at essentially atmospheric conditions, P_T = 760.5 mm. Hg, PO_2 = 152 mm. Hg, P_{N_2} = 596 mm. Hg. Eleven animals in Group III were placed in standard laboratory metabolism cages† for the duration of the experiment.

The usable cage volumes were 2807 and 2698 cc for the plexiglass and metabolism cages, respectively. Cages of approximately the same size were used to provide a control in case the rats' increase in size caused restraint effects toward completion of the experiment.

The total exposure time for all three groups was 64 consecutive days. During this time food (Purina Laboratory Chow) and water were supplied *ad libitum* and consumption of these was measured daily for all animals. Groups II and III were weighed every other day; however, because of the difficulty involved, animals in Group I were weighed only on days 1, 30, 48, 56, and 64 of the experiment. The procedure for weighing the pure oxygen group (Group I) involved transferring the rat at reduced pressure into a special weighing chamber and then back to the original chamber. This procedure was carried on without any change in pressure or gas concentrations.

All chambers were changed and cleaned daily. By means of a transfer tube and gate valve (Figure 2), animals could be transferred into a clean chamber without pressurization of the system to atmospheric conditions.

The variation in over-all pressure control of the chambers was ± 5 mm. Hg. This variation was due to daily fluctuations in atmospheric pressure. Gas concentrations during the experiment was measured by passing the chamber exhaust gas through a paramagnetic oxygen analyzer,* infrared CO_2 analyzer,** and a dew point indicator.*** Gas concentrations were measured at the same pressure as the chamber. A gas flow rate of 800 cc/min. was maintained throughout the experiment for the animals kept in chambers. With this flow rate relative humidity remained between 30-50 per cent and was dependent upon the animal's activity. The source of the water vapor was that expired air. Temperature variation in the chambers was 25 ± 0.4°C. The CO_2 concentration varied between 0.3-1.0 per cent, again depending upon animal activity. Mass spectrometer analysis randomly throughout the experiment gave no indication of contaminants in the chamber gases. A detailed account of the exposure apparatus is in press.[25]

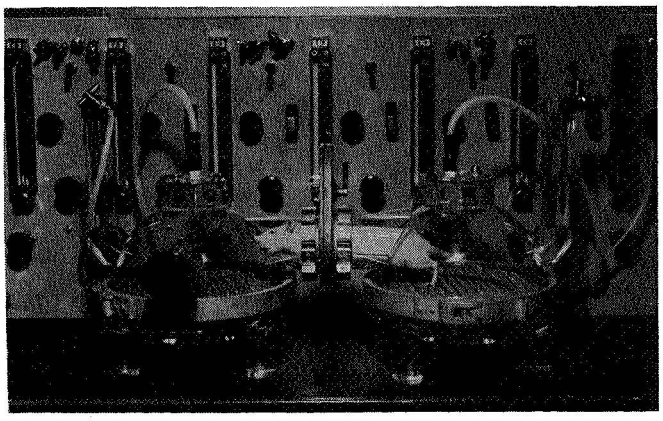

Fig. 2. Exposure chamber transfer system for maintaining animals under constant conditions.

Upon completion of 64 days' continuous exposure, all animals were sacrificed by intraperitoneal injection of 50 mg/kg sodium pentobarbital (Nembutal). This method eliminated traumatic hemorrhage of the lungs incurred by intracranial injection or decapitation.

Immediately following anesthetization, the peritoneal and pericardial cavities were opened and examined. A cardiac puncture was made, 5-6 cc of blood was removed, hematocrit was determined, and the sample was immediately refrigerated at 5°C for selected analysis. Within 3 hours after removing the blood, analyses of red cell glucose-6-phosphate dehydrogenase[4] and glutathione[6] were completed. The following day, WBC, RBC, differential, reticulocyte, and Heinz body counts were performed, osmotic fragility was measured, hemoglobin concentrations were determined, and a particle size distribution plot was obtained by means of a Coulter Counter Model B.

For histological analysis, sections of the lungs, liver, kidneys, heart, thymus gland, adrenal glands, G.I. tract, and CNS were fixed in 10 per cent formalin, 100 per cent ethyl alcohol, or frozen. A complete bone marrow analysis was also performed with smears and whole femur sections.

RESULTS

No unusual behavior or overt indications of distress were noticed during random daily observation of all animals over the entire 64-day period. The animals breathing pure oxygen revealed no paralysis or dyspnea, which other investigators[9,10,29] noted at increased oxygen partial pressures. Following autopsy of the animals microscopic examination of the lungs revealed no pulmonary edema or hemorrhage. All other organs appeared normal.

No significant differences between oxygen exposed and control animals were observed in thymic, cardiac or testicular weights. The mean adrenal weight of the oxygen animals was 42.5 mg, while 43.0 and 48.5 were obtained for control groups II and III, respectively. Lack of significant differences in either absolute or relative $\left(\frac{\text{observed organ weight}}{\text{body weight}} \times 100\right)$ organ weights confirms the absence of restraint stress for the cages used during this experiment.

An analysis of variance, split-plot design (28) showed no differences in body weight increases for the three groups during the 64 days (Figure 3). Food and water consumption for three groups was not significantly different. In general, breathing pure oxygen was not

*Beckman Model No. F-3.

**Mine Safety Appliances Company Model No. 200.

***Cambridge Systems Model No. 990.

†Acme Metal Products.

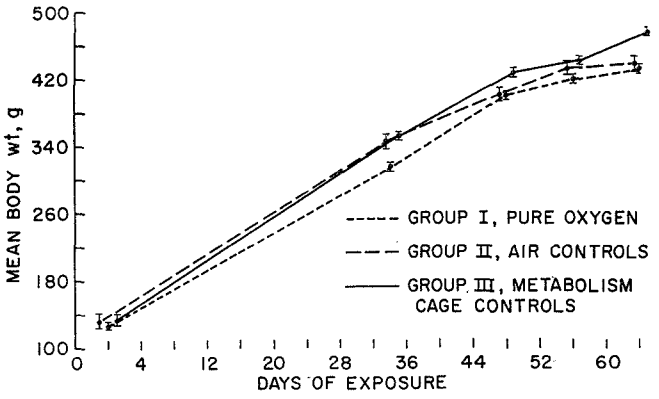

Fig. 3. Growth curves for three groups of animals during the 64 days of the experiment. Points represent arithmetic mean of the group with pooled standard deviations.

found to be detrimental to those metabolic parameters measured for a 64-day extended exposure period at 450 mm. Hg.

Analysis of variance for hematology measurements revealed some differences in the three groups. Those measurements that differed significantly are listed in Table I. Between groups comparisons were made utilizing Scheffe's method.[27] Differences were not observed in total white blood cells, and the differential counts revealed only significant differences in neutrophils and lymphocytes. There was no evidence of Heinz body formation in any of the animals. No differences were found in mean corpuscular volume, mean corpuscular hemoglobin, or mean corpuscular hemoglobin concentration. The particle size distribution plot of blood samples from all the animals also gave no proof of possible changes in blood cell size. Osmotic red cell fragility was not changed with increased oxygen tensions in the pure oxygen group. Bone marrow studies indicated no change in marrow activity with increased oxygen partial pressures.

Terminal histological examination revealed no abnormal tissue in any of the animals. Glycogen distribution, a good index of CNS damage,[21] was normal in the cerebrums and cerebellums of the oxygen exposed group. The distribution was determined by PAS staining followed by amylase digestion. This group was also negative for possible pulmonary tissue changes, although one animal from the oxygen group was afflicted with pneumonia, an illness common in the rat.

DISCUSSION

The results of this study indicated the absence of the oxygen toxicity syndrome. This syndrome has been described[1,9,10,] as being one of severe pulmonary distress characterized by hemorrhage, atelectasis, and extensive edema to the point of filling the lungs and pericardial cavity with fluid.

The possibilities of setting 450-550 mm. Hg as a limit of safety for other animals, particularly man, should be examined with care. Other workers[7,9,29] have described differences in the susceptibility of various animals to an increase in PO_2. Differences have even been noted in the same strain of the rat.[17] Also, other factors reported to effect oxygen toxicity, such as temperature and humidity,[12,13,16] will have to be considered. Increased susceptibility of older animals to oxygen tensions has been observed in our laboratory and is being investigated.

The question of harm from sustained exposure to an increased PO_2 remains to be answered. The present investigation, using a PO_2 slightly below the acute toxic level, revealed no major significant changes in the parameters measured although several observations are worthy of comment. First of all, none of the oxygen animals revealed any overt signs of stress. Those organ weights indicative of possible stress situations and metabolic indices were normal. Apparently, an increase in the inspired oxygen tension from 152 to 450 mm. Hg was not of a magnitude sufficient to produce tissue damage or elicit a major stress response, and any subtle adjustments of the animal, anatomically or physiologically, were not apparent to the investigators. Secondly, no marked changes indicative of major damage were noted in the hematologic measurements. Evidence of oxidative hemolysis described by Helvey, et al., in humans[14] was not observed, nor were the changes in glutathione content sufficient to indicate challenge to the oxidation-reduction capabilities of the red blood cell. No evidence of reticulocytosis was present and the bone marrow studies indicated no change in hematopoiesis. A minor adaptive mechanism may be the depression in hemoglobin content resulting from increased PO_2 exposure which we observed and which has been

TABLE. I. BLOOD MEASUREMENTS FOUND TO BE SIGNIFICANTLY DIFFERENT AFTER 64 DAYS OF CONTINUOUS EXPOSURE TO PURE OXYGEN AT 450 MM HG

	Groups			F Ratio	Probability
	100% Oxygen[12]	Air Controls[6]	Metabolism Cage Controls[11]		
RBC, million/cmm	5.794 ± 0.165*†	6.665 ± 0.196	6.549 ± 0.128	9.0020	$p<0.005$
Neutrophils, %	13.33 ± 3.28†	14.00 ± 2.00	24.46 ± 2.35	4.9252	$p<0.025$
Lymphocytes, %	82.42 ± 3.41†	80.83 ± 2.66	71.18 ± 2.55	4.1836	$p<0.05$
Reticulocytes, %	3.025 ± 0.018†	3.367 ± 0.159	3.355 ± 0.142	3.3959	$p<0.05$
Hemoglobin, mg/100 ml	11.983 ± 0.150*†	12.883 ± 0.083	13.073 ± 0.192	13.5191	$p<0.001$
Hematocrit vol.,%	43.333 ± 0.689†	45.333 ± 0.211	47.300 ± 1.606	3.6308	$p<0.05$
Glutathione, mg/100 ml	36.050 ± 1.147†	37.883 ± 0.768	42.209 ± 1.636	5.9881	$p<0.01$

*Significantly different from air controls at $p<0.05$.

†Significantly different from metabolism cage controls at $p<0.05$.

(Values are expressed as means with standard deviations.)

reported by others.[7,14] Since the red blood cell hemoglobin concentration of the increased oxygen group was not significantly different from the other groups, the drop in hemoglobin was attributed to the decreased RBC content. The decreased RBC content of the pure oxygen group is not surprising considering exposure to an increased oxygen pressure for an extended time period. A slight depression in red blood cell synthesis may also be indicated in that the mean reticulocyte count for the oxygen animals is slightly below that of the other two groups. The fact that glucose-6-phosphate dehydrogenase levels were unchanged would discourage the thought of possible metabolic alterations in the red blood cell subjected *in vivo* to increased PO_2.

In summary, 64 days' exposure to increased oxygen tension produced a few changes for those parameters measured. The possibility exits that little difficulty is encountered until a certain PO_2 threshold level is passed, then acute pulmonary tissue damage develops and the animal succumbs. In general, it may also be concluded that a prolonged increase in the PO_2 did not cause any remarkable changes in the blood variables we measured. These parameters may be relatively resistant until sufficient oxygen tensions are reached to produce the lipid-peroxidation of the red blood cell membrane described by Mengel, et al.[19] Abnormal lipid peroxidation may result only after sufficient oxidant stress develops from an increased oxygen pressure; this phenomenon was not present in the current investigation. On the other hand, longer exposures to pure oxygen environments or increased oxygen pressures may reveal hematological disturbances not encountered in the present study.

ACKNOWLEDGMENT

The excellent assistance of Mr. Eugene Averkin for the statistical treatment of the data is gratefully acknowledged.

REFERENCES

1. Bean, J. W., and Smith, C. W.: Hypophyseal and Adrenocortical Factors in Pulmonary Damage Induced by Oxygen at Atmospheric Pressure. *Am. J. Physiol.* 172:169, 1953.
2. Becker-Freyseng, H., and Clamann, H. G.: Die Wirkung Langdauernder Sauerstoffatmung in Verschiedenen Höhen auf den Menschen. *Luftfahrtmedizin* 7:272, 1942.
3. Behnke, A. R.: High Atmospheric Pressure; Physiological Effects of Increased and Decreased Pressure; Application of These Findings to Clinical Medicine. *Ann. Int. Med.* 13:2217, 1940.
4. Berger, L.: The Semi-quantitative Determination of the Activity in Red Cells or Other Fluids. Tech. Bull. No. 400, Sigma Chemical Co., 1963.
5. Berry, L. J., and Smythe, D. S.: Effect of Pure Oxygen at Reduced Pressure on Metabolic Changes in Mice Living Under Simulated Biosatellite Conditions. Rep. 62.24, USAF School of Aerospace Medicine, Brooks AFB, Texas, 1962.
6. Beutler, E., Duron, O., and Kelly, B. M.: Improved Method for the Determination of Blood Glutathione. *J. Lab. and Clin. Med.* 61:882, 1963.
7. Campbell, J. A.: Prolonged Alterations of Oxygen Pressure in the Inspired Air with Special Reference to Tissue Oxygen Tension, Tissue Carbon Dioxide Tension and Hemoglobin. *J. Physiol.*, 62:211, 1927.
8. Comroe, J. H., Dripps, R. D., Dumke, P. R., and Deming, M.: Oxygen Toxicity: The Effect of Inhalation of High Concentrations of Oxygen for Twenty-four Hours on Normal Men at Sea Level and at a Simulated Altitude of 18,000 feet. *J.A.M.A.*, 128:710, 1945.
9. Cook, S. F., and Leon, H. F.: Survival of C-57 Mice and Squirrel Monkeys in High and Low Pressures of Oxygen. AFMDC-TR-60-21, Holloman AFB, New Mexico, 1960.
10. Dickerson, K. H.: Pathophysiology of Pulmonic Toxicity in Rats Exposed to 100 Per Cent Oxygen at Reduced Pressures. NADC-ML-6403, U. S. Naval Air Development Center, Johnsville, Pennsylvania, 1964.
11. Dines, J. H., and Hiatt, E. P.: Prolonged Exposure of Young Rats to an Oxygen Atmosphere at Reduced Pressure. *J. Appl. Physiol.* 19:17, 1964.
12. Gold, A. J., Silver, E. C., and Hance, H. E.: Influence of Environmental Temperature on the Toxicity of Oxygen. *Aerospace Med.* 35:563, 1964.
13. Grossman, M. S., and Penrod, K. E.: Relationship of Hypothermia to High Oxygen Poisoning. *Am. J. Physiol.* 156:177, 1949.
14. Helvey, W. M., Albright, G. A., Benjamin, F. B., Gall, L. S., Peters, J. M., and Rind, H.: Effects of Prolonged Exposure to Pure Oxygen on Human Performance. Republic Aviation Corp., RAC 393-1 (ARD-807-701), November 1962.
15. Herlocher, J. E., Quigley, D. G., Behar, V. S., Shaw, E. G., and Welch, B. E.: Physiologic Response to Increased Oxygen Partial Pressure. I. Clinical observations. *Aerospace Med.* 35:613, 1964.
16. Hulpieu, H. R., and Cole, V. V.: The Effect of Humidity and Temperature on Oxygen Toxicity. *J. Lab. and Clin. Med.* 29:1134, 1944.
17. Kydd, G. H., Kowalski, L., and McGowan, R.: Lack of Predictability in Rats to Exhibit Chronic Oxygen Poisoning. *Aerospace Med.* 35:634, 1964.
18. MacHattie, L., and Rahn, H.: Survival of Mice in Absence of Inert Gas. *Proc. Soc. Exp. Biol. and Med.*, 104:772, 1960.
19. Mengel, C. E., Kann, H. E., Jr., Lewis, A. M., and Horton, B.: Mechanisms of *in vivo* Hemolysis Induced by Hyperoxia. *Aerospace Med.* 35:857, 1964.
20. Michel, E. L., Langevin, R. W., and Gell, C. F.: Effect of Continuous Human Exposure to Oxygen Tension of 418 mm. Hg for 168 hours. *Aerospace Med.* 31:138, 1960.
21. Miquel, J., Klatzo, I., Menzel, D. B., and Haymaker, W.: Glycogen Changes in X-irradiated Rat Brain. *Acta Neuropathologica* 2:482, 1963.
22. Morgan, T. E., Jr., Ulvedal, F., Cutler, R. G., and Welch, B. E.: Effects on Man of Prolonged Exposure to Oxygen at a Total Pressure of 190 mm. Hg. *Aerospace Med.* 34:589, 1963.
23. Mullinax, P. F., Jr., and Beischer, D. E.: Oxygen Toxicity in Aviation Medicine; an Analysis of Recent Literature. *J. Aviat. Med.* 29:660, 1958.
24. Paine, J. R., Lynn, D., and Keys, A.: Observations on the Effects of the Prolonged Administration of High Oxygen Concentration to Dogs. *J. Thoracic Surgery*, 11:151, 1941.
25. Quattrone, P. D., and Staley, R. W.: Exposure System for Small Animals at Atmosphere and Reduced Pressures. To be published.
26. Roth, E. M.: Space Cabin Atmospheres, Part I—Oxygen Toxicity. NASA SP-47, 1964.
27. Scheefe, H.: The Analysis of Variance. Wiley. (1959). (A Wiley publication in mathematical statistics, pp. 55-87.)
28. Steel, R. G. D., and Torrie, J. H.: Principles and Procedures of Statistics. McGraw-Hill Book Co., pp. 232-249, 1960.
29. Weir, F. W., Bath, D. W., Yevich, P., and Oberst, F. W.: Study of Effects of Continuous Inhalation of High Concentrations of Oxygen at Ambient Pressure and Temperature. *Aerospace Med.* 36:117, 1965.
30. Welch, B. E., Morgan, T. E., Jr., and Clamann, H. G.: Time Concentration Effects in Relation to Oxygen Toxicity in Man. *Fed. Proc.* 22:1053, 1963.